

## The Use of Cardio-Green for Intraoperative Visualization of the Coronary Circulation: Evaluation of Myocardial Toxicity

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*Direct visualization of the extent and adequacy of the coronary blood flow distribution can be performed intraoperatively with an intracoronary injection of indocyanine green dye (Cardio-Green). Because Cardio-Green is potentially toxic to the myocardium, we studied its effects on the mechanical performance of cardiac muscle, using human right atrial trabeculae contracting in vitro. In the first series of experiments, muscles were exposed to 25 mg/ml of Cardio-Green (a concentration 100 times greater than what would be used clinically); no significant alteration in cardiac mechanics was found when evaluated by the force-frequency relationship. To establish the concentration at which Cardio-Green would be toxic, an accumulated dose-response relationship was determined. When a polynomial regression analysis was done, the results best fit a parabolic curve: At high doses, Cardio-Green was found to be negatively inotropic. The estimated concentration that would evoke a 50% decrease in developed force was 41.96 mg/ml. The results show that intracoronary injection of Cardio-Green at clinical doses up to 0.3 mg/ml does not affect myocardial muscle performance. Safe intraoperative use depends on appropriate low doses. (Texas Heart Institute Journal 1987; 14:133-138)*

*Key words: Indocyanine green; coronary circulation; myocardial contraction; aortocoronary bypass*

FOR MANY YEARS, indocyanine green (Cardio-Green), has been used as an indicator dye for measuring blood flow. Previously, we proposed that it be used as a qualitative method for direct intraoperative visualization of the distribution of myocardial flow after coronary artery bypass grafting and especially after

coronary endarterectomy.<sup>1,2</sup> On the basis of our subjective clinical experience with coronary injection of the dye, we felt that Cardio-Green caused no toxic effect at the doses used clinically (0.2-0.3 mg/ml). To objectively assess the myocardial mechanical effects of indocyanine green, we designed the following study, which used

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## MATERIALS AND METHODS

In this project, for which approval was obtained from the Ottawa Civic Hospital Research and Ethics Committee, right atrial appendages were taken from 31 consenting patients (27 men and four women) undergoing open-heart surgery. The patients' ages ranged from 40 to 70 years (mean,  $54.39 \pm 1.66$  years). (Patients under 18 years of age and those undergoing reoperation had been excluded from the study.) Surgical procedures included 29 coronary artery bypass operations, one aortic valve replacement, and one mitral valve replacement. Once the right atrial appendage had been carefully removed, it was stored in heparinized autologous arterial blood and was sent to the muscle laboratory.<sup>3</sup> In each case, one to four trabeculae were removed from the endocardial surface, for a total of 35 samples (Table I).

The specimens were placed between two Lucite clips on a designated muscle stand. The upper clip was attached, via a stainless-steel rod, to a force transducer (UC-2, Gould Statham-Gould, Oxnard, Calif.) whose output was amplified and displayed on a direct writer-recorder (Grass, Model 7 Polygraph, Quincy, Mass.).

The muscles were immersed in an 80-ml bath containing buffered Tyrode's solution, which was aerated with a mixture of 95% oxygen and 5% carbon dioxide and was maintained at a constant temperature of 34°C with a thermoregulated water bath. A pair of platinum plate electrodes was fixed parallel to the sample and was used to field-stimulate the preparation and cause the muscle to contract isometrically. The stimulating pulse was a square wave signal (Grass, Model S88, Quincy, Mass.) 5 to 10 msec in duration, at a voltage 20% greater than that needed to produce a maximal response. The trabeculae were initially equilibrated at a rate of 0.1 pulse/sec for 30 minutes; the rate was then increased to 1.0 pulse/sec for 15 minutes. During this time, the muscles were progressively lengthened until the maximum developed force ( $L_{max}$ , or the peak of the Frank-Starling curve) was reached. Throughout the remainder of the experiment, this physiologic length was maintained.

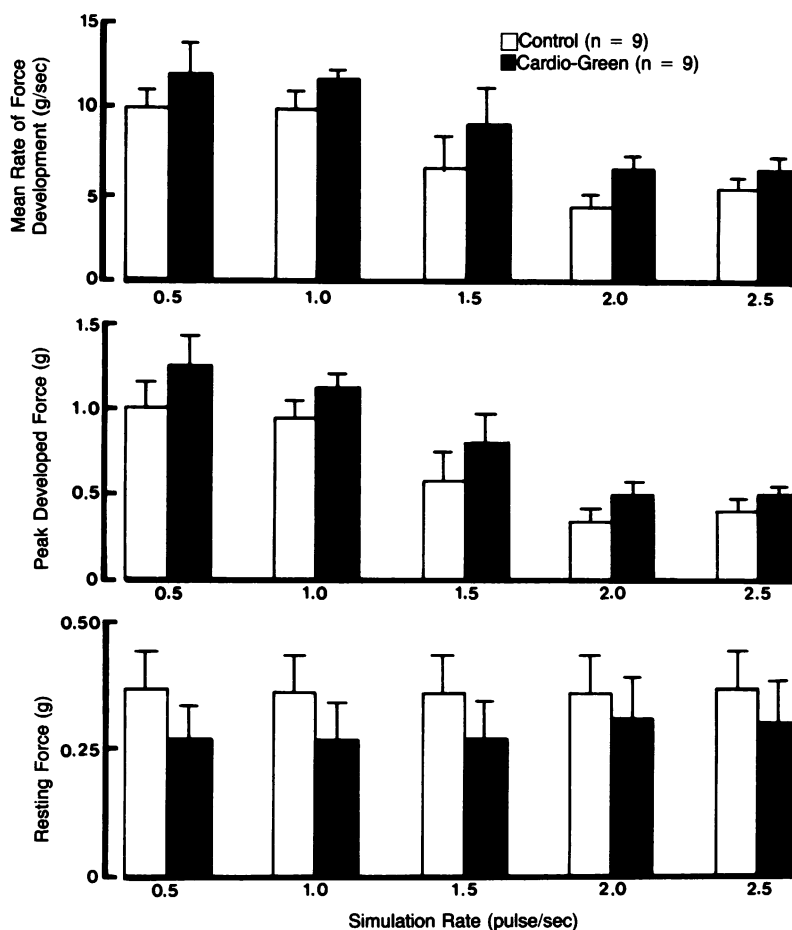
The first series of experiments, using nine test samples and nine control samples, was designed

**TABLE I.** Data Concerning the Trabeculae (mean  $\pm$  SE)

	n = 35
Length (mm)	= $5.97 \pm 0.23$
Weight (mg)	= $5.49 \pm 0.34$
Cross-sectional area (mm <sup>2</sup> )	= $0.88 \pm 0.04$
Resting force (g)	= $0.40 \pm 0.03$
Developed force (g)	= $1.21 \pm 0.06$
Mean rate of developed force (g/sec)	= $12.24 \pm 0.56$

to establish a mechanical response to a specific concentration of indocyanine green (Cardio-Green, Hynson, Wescott and Dunning, Baltimore, Md.). In these experiments, isometric parameters included developed forces (DF) and resting forces (RF), as well as the mean rate of force development (MRDF). In the nine test samples, the stimulation rate was incrementally changed to 0.5, 1.0, 1.5, 2.0, and 2.5 pulse/sec. With each change in rate, the muscle was allowed to equilibrate for 2 minutes before the isometric parameters were measured. The Tyrode's solution was then removed and was replaced by additional Tyrode's solution in which Cardio-Green had been dissolved to a concentration of 25 mg/ml, a value arbitrarily chosen to be 100 times greater than the usual clinical concentration. After a further 10 minutes of equilibration, the stimulation rates were again changed sequentially, and the parameters were measured at each frequency. In each case, the two tests (determination of frequency-force relationship in Tyrode's solution, with and without Cardio-Green) took approximately 20 minutes to complete. In the nine control samples, Cardio-Green was not added in the second half of the experiment, but a second frequency-force measurement was performed as outlined above.

In a second series of experiments, using eight test samples, an accumulated dose-response curve for Cardio-Green was determined. Initial experiments were performed to determine the dose of Cardio-Green that would lead to a complete loss of force within the first hour after stabilization, so as to minimize the loss in force owing to the natural decay of this preparation. Accordingly, in eight test samples, Cardio-Green (100 mg/ml) was continuously added to the Tyrode's solution at a rate of 0.97 mg/min, with a maximum concentration of 96.6 mg/ml



**Fig. 1** Isometric mechanical responses (mean  $\pm$  SEM) to 25 mg/ml of indocyanine green (Cardio-Green) in human right atrial trabeculae contracting in vitro at various stimulation rates.

being reached within the first hour.

In an earlier study, we demonstrated that, with time, this preparation exhibits a natural decay in isometric DF.<sup>3</sup> Therefore, nine control samples were left to contract in Tyrode's solution alone for 120 minutes. Measurements of DF were made every 5 minutes.

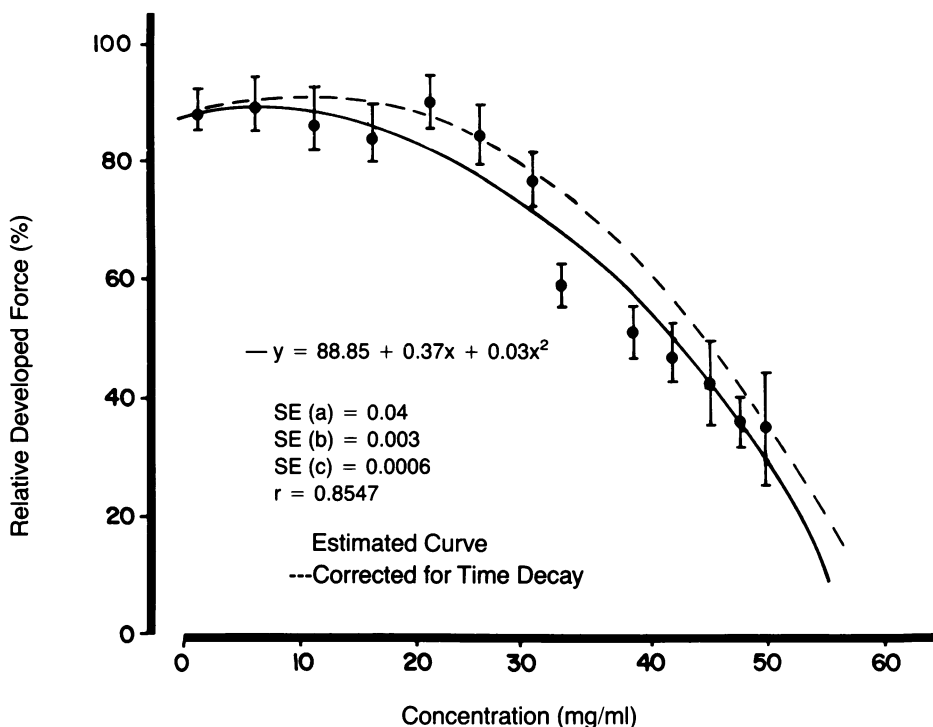
A one-way analysis of variance was used to assess the differences between the response characteristics of all four groups of trabeculae. Statistical analysis of the frequency-force results was performed, using a paired *t* test to compare the control and the non-control samples. The results of the dose-response experiments were assessed according to a polynomial regression analysis. Owing to the natural decay of the preparation with time, it was necessary to correct the results: Because the concentration of Cardio-Green within the bath was known at any given time, the relative DF was corrected by dividing

by the relative decrease in the DF of the control muscles at the same time. A *p*-value of less than 0.05 was considered to be significant.

## RESULTS

The one-way analysis of variance showed no significant difference between the trabeculae's characteristics in any of the four groups. In our first series of experiments with Cardio-Green, the mechanical effects of one concentration (25 mg/ml) were evaluated in nine muscles, and the results were compared to those of the control group (*n* = 9) (Fig. 1). When muscles contracting in Cardio-Green were compared with control samples at similar stimulation rates, there were no significant differences in DF, RF, or MRDF.

Having shown that concentrations of 25 mg/ml do not affect cardiac performance in vitro, we



**Fig. 2** Accumulated dose-response relationship between relative change in developed force (%) and concentration of indocyanine green (mg/ml) in eight human atrial trabeculae. The solid circles indicate the mean relative change in developed force associated with specific concentrations of indocyanine green ( $\pm$  SEM). The solid line is a least squares fit to the uncorrected data, whereas the dotted line has been corrected for the natural decay in developed force with time.

felt it necessary to establish a concentration that would alter the muscles' performance. Accordingly, we derived accumulated dose-response relationships by using eight trabeculae (Fig. 2). A polynomial regression analysis was performed to derive the curve of best fit, which was a parabola with the equation:

$$y = 88.85 + 0.37x - 0.03x^2$$

where  $y$  is relative DF (%) and  $x$  is concentration of Cardio-Green (mg/ml).

To monitor the decay in developed force, we maintained nine trabeculae under control conditions for 120 minutes (Fig. 3). The linear regression analysis equation was:

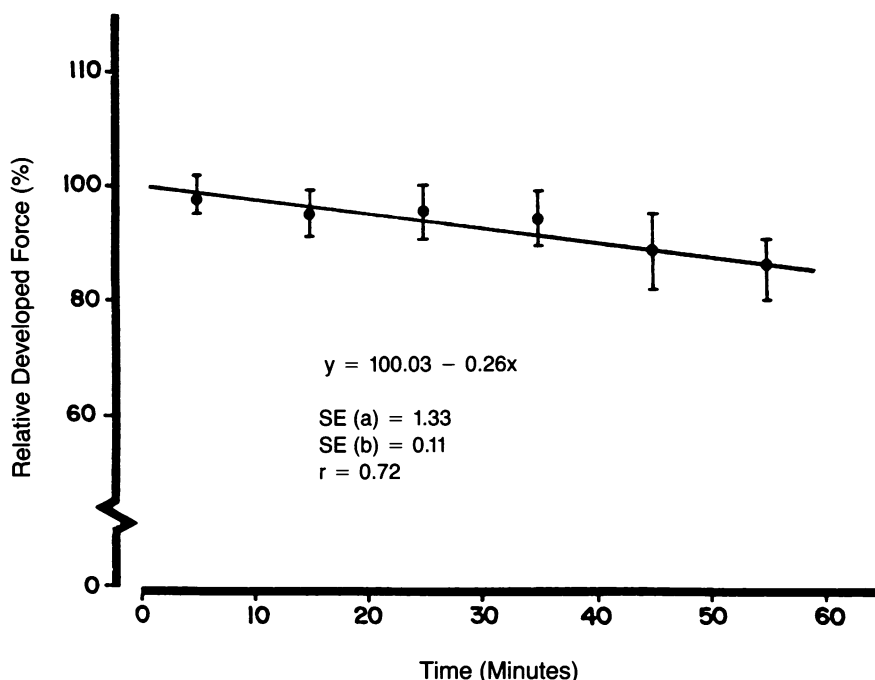
$$y = 100.03 - 0.26x$$

where  $y$  is relative DF (%) and  $x$  is time (minutes). This equation was used to calculate changes in DF at different times in order to correct the dose-response curve for time decay.

The resultant curve is shown in Figure 2. The estimated concentration at which a 50% depression of DF would occur was 41.96 mg/ml.

## DISCUSSION

Injection of a dilute solution of Cardio-Green into a coronary artery graft (either with or without endarterectomy) allows easy, quick intraoperative visualization of the run-off from bypass grafts and endarterectomies.<sup>1,2</sup> After assessing the adequacy of myocardial revascularization, the surgeon can immediately correct any technical problems with the anastomosis or can further extend the endarterectomy. Since 1979, we have used this technique in more than 1,500 cases of coronary artery bypass, with or without endarterectomy. The injectable solution is made by dissolving Cardio-Green powder in 80 to 100 ml of saline, for a final concentration of 0.2 to 0.3 mg/ml. The solution is then injected through the proximal end of the vein graft after distal anastomosis. The extent of



**Fig. 3** Average decay in relative developed force with time, seen in nine human right atrial trabeculae contracting at 1.0 Hz in 34° C Tyrode's solution and aerated with 95% oxygen and 5% carbon dioxide. The closed circles indicate average data points ( $\pm$  SEM).

myocardial perfusion can be evaluated by observing the green myocardial blush. This method has been particularly useful in evaluating the quality of distal core endarterectomies.

Indocyanine green is a water-soluble, tricarbo-cyanine dye that has been used extensively in determining cardiac outputs and other organ blood flows.<sup>4</sup> The dye contains a small amount of sodium iodide and, in individuals who are sensitive, may lead to rare allergic reactions. It is generally safe, however, and has a high median lethal dose (approximately 50 to 80 mg/kg in experimental animals).<sup>5</sup> For these reasons, we have believed that it should be safe for injection into the coronary vasculature, although this application has not been cited by the manufacturer. Because Renografin and other dyes have been shown to cause negative inotropic effects,<sup>6</sup> it is reasonable to question the biological inertness of indocyanine green and its potential for impairing myocardial performance.

Previous studies have shown that the frequency-force relationship is species specific and that it can be used as a measure of myocardial

mechanical integrity.<sup>7,8</sup> With this technique, we found no significant difference between control and noncontrol groups, even though the dye's concentration (25 mg/ml) was 100 times the normal clinical dose. Because a fixed dose of indocyanine green showed no negative inotropic effect, we constructed an accumulated dose-response curve to identify the concentration of drug that would be cardiotoxic. The dose-response curve showed a parabolic relationship, with minimal deterioration in muscle performance within the clinically applicable dose range.

In conclusion, we have found the intraoperative use of indocyanine green to be of great assistance in assessing the distribution of myocardial blood flow. On the basis of the foregoing study, we recommend that doses greater than 0.3 mg/ml not be used.

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## REFERENCES

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1. Keon WJ. Manual coronary endarterectomy and revascularization: Improving techniques and results. *Ann Thorac Surg* 1981; 32:427.
2. Keon WJ, Akyurekli Y, Bedard P, Brais M, Goldstein W, Beanlands DS. Coronary endarterectomy: An adjunct to coronary artery bypass grafting. *Surgery* 1979; 86:859.
3. Keon WJ, Taichman GC, Mainwood G. Human atrial trabeculae contracting in vitro: An exploitable model. *Can J Surg* 1984; 27:372.
4. Symposium on diagnostic application of indicator-dilution technics. *Proc Staff Mtg Mayo Clin* 1957; 32:463.
5. Cardio-Green: Product monograph. Baltimore, Hynson, Wescott and Dunning, July 1978.
6. Cohen MV. The effects of intracoronary Renografin injection on coronary flow and distribution in dogs following coronary occlusion. *Basic Res Cardiol* 1983; 78:364.
7. Blinks JR, Koch-Weser J. Analysis of the effects of changes in rate and rhythm upon myocardial contractility. *J Pharmacol Exp Ther* 1961; 134:373.
8. Anderson PAW, Manning A. Frequency-force relationship: A basis for a new index of cardiac contractility. *Arch Res* 1973; 33:665.